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EFFECT OF SOME SOIL HERBICIDES OF THE VEGETATIVE HABITS AND PIGMENT CONTENT OF *PRUNUS DOMESTICA* 'WANGENHEIMS' PLUM ROOTSTOCK UNDER *IN VITRO* CONDITIONS

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Abstract

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The aim of the present study was to investigate the effect of application of the soil herbicides pendimethalin and napropamide on the vegetative habits and the pigment content in the leaves of *Prunus domestica* **'***Wangenheims'* plants under model *in vitro* conditions. The soil herbicides were applied when the roots were 2-3 mm in length. The herbicide solution was applied under sterile conditions as a film on the nutrient medium surface. Five variants in 3 replicates were set: Control (untreated) – destillated water; **P400** - Pendimethalin – Stomp 33 EC – 4.0 l ha^{-1}; **P600** - Pendimethalin – Stomp 33 EC – 6.0 l ha^{-1}; **N400** - Napropamid - Devrinol 4F – 4.0 l ha^{-1}; **N600** - Napropamid - Devrinol 4F – 6.0 l ha^{-1}. Visual observations on plant development and the appearance of external symptoms of phytotoxicity (chlorosis, necrosis, plant withering) were carried out in dynamics on the 7th, 14th and 21st day after treatment. Data about the stem height, mean length of the roots, relative growth rate (RGR), as well as the content and ratio of the photosynthetic pigments were reported on the 21st day. Thesymptoms of phytotoxicity (necrosis in the root-formation area) appeared on the 14th day after treatment with napropamide. On the 21st day after treatment with pendimethalin, chlorosis in the leaves of the *in vitro* plants and obvious growth suppression were established. The active substance napropamide exerted a poorer inhibiting effect on the plant growth in height compared to pendimethalin. Napropamide at concentration 6.0 $l.ha^{-1}$ had the strongest depressing effect on root growth of the *in vitro* plants of the studied plum rootstock.

Key words: in vitro, soil herbicides, phytotoxicity, rootstock
Abbreviations: Relative Growth Rate - RGR; BAP - 6-benzylaminopurine; IBA - indole-3-butyric acid; DMRT - Duncan's Multiple Range Test

Introduction

Production of grafted fruit tree planting material is an important starting element for the development of modern fruit growing. The wide use of seedling rootstocks in the nurseries is typical of many South-East European countries, including Bulgaria. Herbicide application for weed control in the production process is risky for causing phytotoxicity on non-target plants because of the probable direct contact between the sprouting germ and the soil herbicide. Many authors have reported that some nonselective herbicides such as 2,4-D, glyphosate, chlorsulfuron or trichloroacetate may cause severe damage to crops by inducing leaf necrosis, an increase in stomatal resistance, inhibition of shoot growth, decrease in germination, accumulation of reactive oxygen species or reduction of net photosynthesis (Bhatti et al., 1998; Radetski et al. 2000).

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There are scarce data in literature about the effect of soil and leaf herbicides on the growth of fruit tree species used as seedling rootstocks. The effect of applying soil herbicides was studied in yellow plum seedling rootstocks (Porterfield et al, 1993; Wazbinska, 1997; Kaufman and Libek, 2000; Rankova, 2004), in peach (Arenstein, 1980; Lange, 1987; Abdul et al., 1998; Lourens et al., 1998; Rankova, 2004), in wild cherry (Clay, 1984; Porterfield, 1993), in apricot (Arenstein, 1980; Mitchell, 1989). Different, species-inherent responses were established – from lack of phytotoxicity and obtaining good quality rootstocks suitable for grafting to very strong toxicity after applying some active substances contained in the herbicides, followed by dying of the plants. That is why preliminary studies are needed to estimate the effect of different herbicides on the vegetative habits of the rootstocks.

Model studies under *in vitro* conditions on phytotoxicity caused by soil herbicides have also been carried out. Different effects of the soil herbicide pendimethalin on the development of the embryo root depending on its length at the moment of treatment were established in yellow plum embryos (Gercheva et al., 2002). Results were obtained about the inhibiting effect or the lack of visual symptoms of phytotoxicity of the soil herbicides napropamide, pendimethalin and terbacil in some vegetative rootstocks (GF-677, MM 106) under *in vitro* conditions (Rankova et al., 2006a, b; 2009).

Wangenheims plum rootstock is a perspective seedling rootstock, for which it was established that it controlled tree size, increased tree prolificacy and stimulated earlier blooming and fruit ripening (Blazek et al., 2004; Grzyb and Sitarek, 2006). Until now, we do not have data available about the effect of the herbicide application in the process of its growing.

The aim of the present study was to investigate the effect of application of the soil herbicides pendimethalin and napropamide on the vegetative habits and the pigment content in the leaves of rooted plants of *Wangenheims* under model *in vitro* conditions.

Material and Methods

The studies were carried out at the Laboratory of Biotechnologies within the Fruit-Growing Institute - Plovdiv.

Plant Material

In vitro rooted plants of the plum rootstock *Wangenheims* were used for the experiment. Micropropagation was carried out in the basic nutrient medium MS (Murashige and Skoog, 1962), supplemented with 2,5 μM BAP, 0,005 μM IBA, 30 $g.l^{-1}$ sucrose, 6,5 $g.l^{-1}$ agar, pH 5.6 (before autoclaving).

The microplants (2 cm in height) were rooted in a nutrient medium of ¼ strength of macroelements MS, full-strength of microelements and vitamins by MS, 1.5 μM IBA, 20 $g.l^{-1}$ sucrose, 6,5 $g.l^{-1}$ agar, pH 5,6.

The soil herbicides were applied under *in vitro* conditions when the roots were 2-3 mm in length. The herbicide solution was applied under sterile conditions as a film on the nutrient medium surface. The tested concentrations followed the recommendations of the companies for use in fruit nurseries, the rates being calculated proportionally according to the surface area of the cultivation vessel. Five variants in 3 replicates were set (10 plants per replicate):

1. Control (untreated) – destillated water - 4.0 $l.ha^{-1}$;
2. P400 - Pendimethalin – Stomp 33 EC – 4.0 l ha^{-1};
3. P600 - Pendimethalin – Stomp 33 EC – 6.0 l ha^{-1};
4. N400 - Napropamid - Devrinol 4F – 4.0 l ha^{-1};
5. N600 - Napropamid - Devrinol 4F – 6.0 l ha^{-1}.

The plants were cultivated in a growth chamber at a temperature 22±2°C and a photoperiod 16/8 hours (40 μmol m^{-2} s^{-1} PPFD).

Visual observations on plant development and the appearance of external symptoms of phytotoxicity (chlorosis, necrosis, plant withering) were carried out in dynamics on the 7th, 14th and 21st day after treatment.

On the 21st day, the following biometric characteristics were reported: stem height (mm), mean length of the roots (mm), relative growth rate (RGR), as well as the content and ratio of the photosynthetic pigments. RGR was calculated from the equation:

$$RGR = (\ln W_2 - \ln W_1)/ (t_2 - t_1),$$

where W_1 and W_2 are plant weight at time t_1 and t_2, respectively.

The content of the photosynthetic pigments was determined spectrophotomethrically in 80 % acetone extract and was calculated following the equations of Lichtenthaler and Wellburn (1983).

Data were analyzed by analysis of variance and the means were separated using the Duncan's multiple range test (DMRT), ($P < 0.05$).

Results and Discussion

The results obtained showed the different effects of the soil herbicides on the development of the *in vitro* plants, expressed in the appearance of the external symptoms of phytotoxicity, as well as in different periods of the symptom occurrence. On the 7th day, visual symptoms of phytotoxicity were not observed on the plant leaves and stems. The vegetative tips of the explants of the variants treated with pendimethalin (variants P400, P600) and napropamide (var. N400, N600) were fresh, actively growing, without growth suppression. In both variants treated with Devrinol 4F (var. N400, N600), an obvious depression of the root growth and necrosis on their tips were registered. In the variants treated with pendimethalin the depression of the root growth was very slightly expressed with no occurrence of necrosis being established.

On the 14th day in both variants treated with pendimethalin (var. P400, P600), slightly expressed chlorosis in the leaves, growth suppression and delayed root growth were observed. In the variants 4 and 5, the leaves also slightly turned yellowish and very strong necrosis in the roots and obvious growth depression were registered. The vegetative tips of the explants of those variants were fresh and no depression in their growth in height was established.

On the 21st day the symptoms of phytotoxicity were very strong (Figure 1). The plants of the variants P400 and P600 showed obvious growth suppression, chlorosis in the leaves and the vegetative tips were totally withered. The stems withered and turned brownish. The plants treated with two different rates of napropamide (var. N400 and N600) did not show symptoms of phytotoxicity in the leaves and stems. The vegetative tips were fresh, green and no suppression of growth in height was observed. However, necrosis and obvious suppression of root growth were reported.

The results of the biometric analysis confirmed the different effects of the soil herbicides on the growth and development of the *in vitro* plants. The two tested herbicides significantly suppressed microplants growth. Relative growth rate and stem growth in height were affected (Figures 2 and 3). Comparatively slight depressing effect on the growth of the plantlets in height was established in both variants treated with napro-

Fig. 1. Effect of the soil herbicides on rooted plants of *Wangenheims.* Control – destillated water; P400 - pendimethalin – 4.0 $l.ha^{-1}$; P600 - pendimethalin – 4.0 $l.ha^{-1}$; N400 napropamide – 4.0 $l.ha^{-1}$; N600 - napropamide – 6.0 $l.ha^{-1}$

pamide (Var. N400 and N600), (Figure 2), while the plants treated with pendimethalin (var. P400, P600) had a strongly suppressed growth rate. Similar habits of a depressing effect on plant height, caused by the treatment with pendimethalin, were observed in previous studies under the conditions of sand culture (Rankova Z., 2004; 2006). Those habits could be explained by the physical basis of selectivity of the active substance and its ability of exerting a depressing effect when in direct contact with germinating seeds or meristems of growing roots (Tonev, 2000).

A significant effect of the herbicides on the formation of new roots was not established, which was probably due to the experimental methods used – treatment of the already rooted *in vitro* plants. In our previous studies on the effect of pendimethalin and napropamide on root formation of non-rooted *in vitro* plants of the same rootstock, a depressing effect of both herbicides was established in the process of rooting of the plantlets (Rankova et al., 2006a).

In the present study, an obviously expressed effect of the herbicides on root growth was also detected (Figure 4). The strongest depressing effect on that characteristic was established in the plants treated with the higher rate of napropamide (var. N600). When the lower rate of napropamide was applied (var. N400), root growth suppression was more weakly expressed. In both variants with pendimethalin applied (var. P400, P600), the established inhibition of root growth was the same. The differences to the control were statistically highly significant.

That could be explained by the mechanism of action of the active substance of the herbicides, which was expressed in blocking the root growth of susceptible plants (Fetvedzhieva N., 1994; Tonev, 2000).

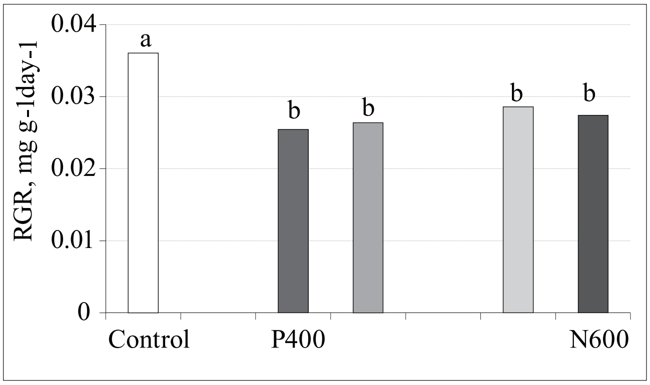

Fig. 2. Effect of the soil herbicides on the relative growth rate (RGR) of the Wangenheims plantlets on dry weight basis, for the plants in a cultivation vessel. Control – destillated water; P400 - pendimethalin – 4.0 l.ha^{-1}; P600 - pendimethalin – 4.0 l.ha^{-1}; N400 napropamide – 4.0 l.ha^{-1}; N600 - napropamide – 6.0 l.ha^{-1}.
Different letters within each column indicates significant difference (P<0.05) by DMRT

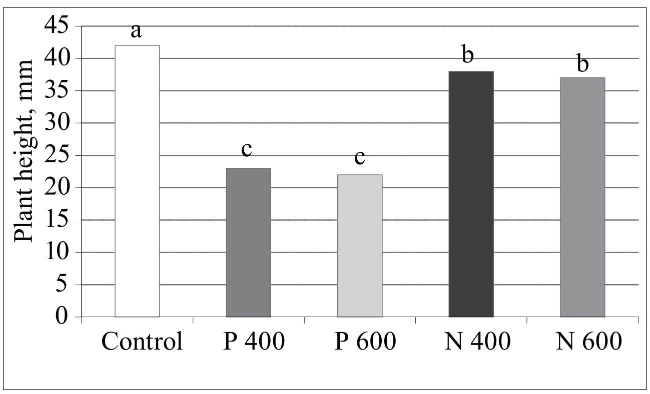

Fig. 3. Effect of the soil herbicides on plant height (mm). Control – destillated water; P400 - pendimethalin – 4.0 l.ha^{-1}; P600 - pendimethalin – 4.0 l.ha^{-1}; N400 napropamide – 4.0 l.ha^{-1}; N600 - napropamide – 6.0 l.ha^{-1}.
Different letters within each column indicates significant difference (P<0.05) by DMRT

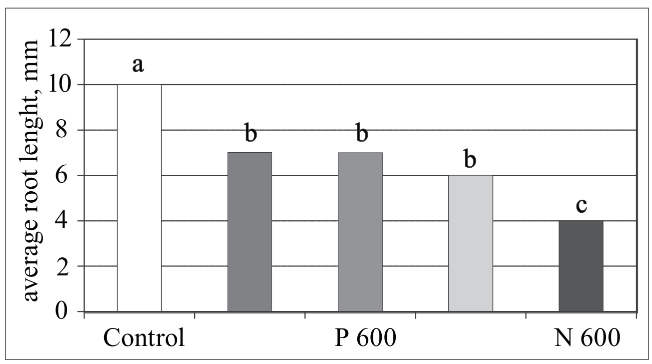

Fig. 4. Effect of the soil herbicides on the root length. Control – destillated water; P400 - pendimethalin - 4.0 l.ha^{-1}; P600 - pendimethalin - 4.0 l.ha^{-1}; N400 napropamide - 4.0 l.ha^{-1}; N600 - napropamide – 6.0 l.ha^{-1}.
Different letters within each column indicates significant difference (P<0.05) by DMRT

The ratio of dry weight to fresh weight at the beginning of the experiment averaged 0.1729 and at the end of the experiment the dry to fresh weight ratios were 0.1589 in the control. This ratio was similar in herbicide treated plants and there was not any significant difference among the treatments with exception of variant P400, which was lower than the control (Table 1).

Data about the content of photosynthetic pigments confirmed the observed symptoms of chlorosis (Fig. 5). The content of chlorophyll a in the plants treated with pendimethalin (P400, P600) was almost twice lower compared to the control. In the variants treated with napropamide (N400, N600) there were no significant differences in the chlorophyll content in comparison with the control plants. Reduced content of carotenoids was also reported in all the treated plants. The ratio between the major groups of photosynthetic pigments was also disbalanced (Figure 6).

Analogous data were obtained about the effect of the herbicide flumioxazin on the growth and photosynthesis of grapevines under *in vitro* conditions, in which reduced chlorophyll content was detected in the leaf tissues and a considerable decrease of the total biomass, of the photosynthetic gas-exchange and the content of carotenoids in the leaves was reported (Saladin et al., 2003). The results obtained showed that the *in vitro* plants could be used as a test for evaluation of the effect of the soil herbicides on the cultural plants.

Table 1
Effect of the soil herbicides on the relative share of the botanical organs of the 'Wangenheims' plantlets on dry weight basis.

Variants	DW/FW %	Relative share of the botanical organs (%) on dry weight basis		
		leaves	stems	roots
Control	15.89 a	57.83 b	32.24 b	9.92 b
P400	13.24 b	61.26 a	28.69 b	10.06 b
P600	15.01 a	56.47 b	29.8 b	13.73 a
N400	16.61 a	56.39 b	36.41 a	7.21 c
N600	15.43 a	58.76 ab	32.86 ab	8.38 bc

Control – destillated water; P400 - pendimethalin – 4.0 $l.ha^{-1}$; P600 - pendimethalin – 4.0 $l.ha^{-1}$; N400 napropamide – 4.0 $l.ha^{-1}$; N600 - napropamide – 6.0 $l.ha^{-1}$. Different letters within each column indicates significant difference ($P<0.05$) by DMRT

Obvious indications were obtained about the existing stress response in the microplants of the plum rootstock Wangenheims (*Prunus domestica*) treated with herbicides. The active substance napropamide had a depressing effect; however, it was less expressed compared to the inhibiting effect of pendimethalin on the growth of the apical meristems. The strongest depressing effect on the root growth was established in the plants treated with the higher rate of napropamide.

The results of the investigation confirmed the necessity of a detailed study on the effect of different active substances on the concrete genetic types. The results ob-

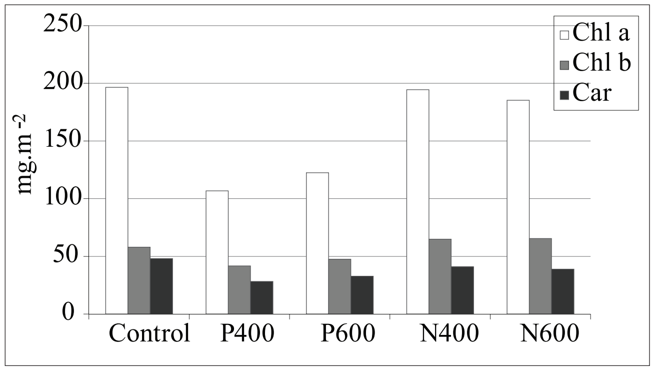

Fig. 5. Effect of the soil herbicides on the content of photosynthetic pigments. Control – destillated water; P400 - pendimethalin - 4.0 $l.ha^{-1}$; P600 - pendimethalin - 4.0 $l.ha^{-1}$; N400 napropamide - 4.0 $l.ha^{-1}$; N600 - napropamide – 6.0 $l.ha^{-1}$.

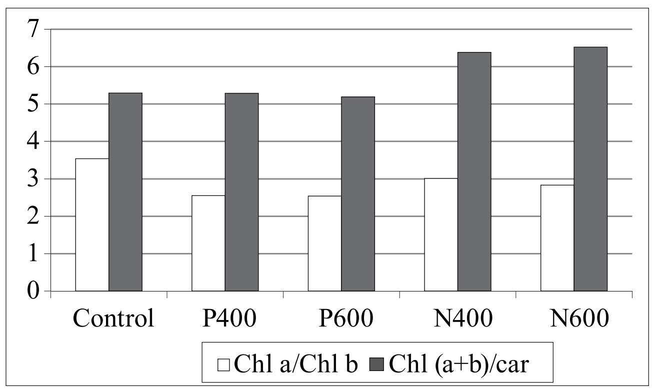

Fig. 6. Effect of the soil herbicides pendimethalin and napropamide on the ratio between the photosynthetic pigments. Control – destillated water; P400 - pendimethalin - 4.0 $l.ha^{-1}$; P600 - pendimethalin - 4.0 $l.ha^{-1}$; N400 napropamide - 4.0 $l.ha^{-1}$; N600 - napropamide – 6.0 $l.ha^{-1}$.

tained gave the grounds to recommend the application of the lower tested rates of the soil herbicides Stomp and Devrinol when growing the vegetative plum rootstock Wangenheims (*Prunus domestica*) in the nursery and in plants with well-developed root systems.

Conclusions

The external symptoms of phytotoxicity (necrosis in the root-formation area) appeared on the 14th day after treatment with napropamide. On the 21st day after treatment with pendimethalin, chlorosis in the leaves of the *in vitro* plants and obvious growth suppression were established. The active substance napropamide exerted a poorer inhibiting effect on the plant growth in height compared to pendimethalin. Napropamide at concentration 6.0 $l.ha^{-1}$ had the strongest depressing effect on root growth of the *in vitro* plants of the studied plum rootstock.

References

Abdul, B., K. Mohammad and J. Tasleem, 1998. The effect of chemical weed control in peach nursery on germination and seedling growth. Sarhad *Journal of Agriculture*, **14** (1): 43-47.

Arenstein, Z., 1980. The control of annual weeds in young and matute orchards by means by terbutryne and terbutryne/simazine mixture. British crop protection conference of weeds, Nottingam, *Proceedings*, **1:** 159 – 163.

Bhatti, M. A., A. S. Felsot, R. Parker and G. Mink, 1998. Leaf photosynthesis, stomatal resistance, and growth of wine grapes (*Vitis vinifera* L.) after exposure to simulated chlorsulfuron drift. *J. Environ. Sci. Health,* **B 33**: 67–81.

Blazek, J., R. Vavra and I. Pistekova, 2004. Orchard performance of new plum cultivars on two rootstocks in a trial at Holovousy in 1998–2003. *Hort. Sci. (PRAGUE)*, **31** (2): 37–43.

Clay, D. V., 1984. Evaluation of the tolerance of cheery and plum trees to root application of herbicide using a sand culture method. *Aspects of Applied Biology. Assoc. of Applied Biologist*, **8**: 75 – 86.

Fetvadzhieva, N., 1973. Weed Control. *Zemedelie,* Sofia, 234 pp. (Bg).

Gercheva, P., Z. Rankova and K. Ivanova, 2002. *In vitro* Test System for Herbicide Phytotoxicity on Mature Embryos of Fruit Species. *Acta Horticulturae,* **577**: 333- 336.

Grzyb, Z. B. and M Sitarek., 2006. The influence of different rootstocks on the growth, yield and fruit quality of plum tree cv Dabrovice Prune planted in exhausted soil. *Scientific works of the Lithuanian Institute of Horticulture and Lithuanian university of Agriculture,* **25** (3): 292-295.

Kaufman, E. and A. Libek, 2000. The application of herbicides in a fruit – tree nursery. Proceedings of the International Conference Fruit Production and Fruit Breeding, Tartu, Estonia, pp. 61- 64.

Lange, A. H., 1987. Comparative phytotoxicity studies in trees and vines. Proceedings 39 th annual California weed conference, pp. 210 – 212.

Lichtenthaler, H. and A. Wellburn, 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans,* **603**: 591-592.

Lourens, A.F., A. Lange and F. Calitz, 1989. Phytotoxicity of pre-emergence herbicides to peach seedlings (*Prunus persica*). *South African Journal of plant and soil*, **6**(2): 97–102.

Mitchell, R .B. and R. J. Abernethy, 1989. The effect of weed removal on the growth of young apricot trees. (Proc.40 th New Zealand Weed and Pest control conf. Nelson, Aug.11-13), *Palmerston North*, pp. 209 -212.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and biossays with tobacco tissue cultures. *Physiol. Plant*, **15**: 473-497.

Porterfield, J. D., J. D. Odell and G. R. Huffman, 1993. Effects of DCPA/ napropamide herbicide tank mix on germinant of seven hardwood species in tree nursery beds. *Tree Planters Notes,* **44** (4): 149-153.

Radetski, C. M., S. Cotelle and J. F. Ferard, 2000. Classical and biochemical endpoints in the evaluation of phytotoxic effects caused by the herbicide trichloroacetate. *Environ. Exp. Bot.,* **44:** 221–229.

Rankova, Z., 2006. Effect of some soil herbicides on the vegetative habits of mahaleb cherry (*Prunus machaleb* L.) seedling rootstocks. *Bulgarian Journal of Agricultural Science,* **12:** 429-433.

Rankova, Z., 2004. Study on the effect of some soil herbicides on the vegetative habits of yellow plum and peach seedling rootstocks, *PhD Thesis*, Fruit growing Institute- Plovdiv, pp. 156 (Bg).

Rankova, Z., L. Nacheva, P. Gercheva and V. Bozkova, 2006b. Vegetative habits of plum rootstock Wangenheims after treatment with terbacil under in vitro conditions. VI National Conference “Ecology and helth” Plovdiv, May 2006. pp. 339-344.

Rankova, Z., L. Nacheva, K. Zapryanova, P. Gercheva and V. Bozkova, 2006a. Effect of soil herbicides napropamid and pendimethalin on rooting and growth of the vegetative plum rootstock Pr. domestica Wangenheims under *in vitro* conditions*. Journal of Mountain Agriculture on the Balkans,* **9** (3): 349-359.

Rankova, Z., L. Nacheva and, P. Gercheva, 2009. Growth Habits of the Vegetative Apple Rootstock MM 106 after Treatment with some Soil Herbicides under i*n vitro* Conditions. *Acta Horticulturae,* **825**: 49-54.

Saladin, G., C. Magne and C. Clement, 2003. Stress reactions in *Vitis vinifera* L. following soil application of the herbicide flumioxazin*. Chemosphere*, **53:** 199–206.

Tonev, T., 2000. Handbook of integrated weed control and culture of farming. Book 2, Higher Institute of Agriculture, Plovdiv, pp. 275, pp. 126-127 (Bg).

Wazbinska, J., 1997. Technological improvement of generative cherry plum rootstocks, one-year Wegierka Lowicka plum trees and apple seedlings. Acta. Academiae Agriculturae ac Technicae. *Olstenensis Agricultura,* **64:** 107-110.

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